

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)**ScienceDirect**

Procedia Chemistry 9 (2014) 151 – 157

**Procedia**  
Chemistry

International Conference and Workshop on Chemical Engineering UNPAR 2013, ICCE UNPAR 2013

## The Effect of Alkaline Addition in Hydrothermal Pretreatment of Empty Fruit Bunches on Enzymatic Hydrolysis Efficiencies

Siti Aisyah MS<sup>a\*</sup>, Yoshimitsu Uemura<sup>a</sup>, Suzana Yusup<sup>b</sup>,<sup>a</sup>Center for Biofuel & Biochemical Research, Universiti Teknologi PETRONAS, Bandar Seri Iskandar, 31750 Tronoh, Perak.<sup>b</sup>Chemical Engineering Department, Universiti Teknologi PETRONAS, Bandar Seri Iskandar, 31750 Tronoh, Perak.\* Corresponding author. Tel.: +605-3687645; fax: +605-3687649. E-mail address: [aisyah.msaman@gmail.com](mailto:aisyah.msaman@gmail.com).

### Abstract

In general, lignocellulosic biomass contains three major components, namely lignin, hemicellulose and cellulose which are the polymers of C5 and C6 sugars. Thus, there is potential to utilize of this biomass for bioethanol production. The hydrolysis of cellulose into glucose was difficult due to the more fibrous nature and thus inhibit enzyme penetration into the cellulose. In order to solve this problem, hydrothermal pretreatment can be used for breaking the bonds within the lignin structure and increase the accessibility of enzyme into the cellulose. In this study, the effect of chemical addition, sodium hydroxide (NaOH) and calcium oxide (CaO) in hydrothermal pretreatment at 180 °C and 30 minutes reaction time of palm oil empty fruit bunches (EFB) on the enzymatic hydrolysis efficiencies was investigated. The enzymatic hydrolysis of hydrothermally pretreated EFB give the highest concentration of glucose at 0.67 g/L while the hydrothermally pretreated of EFB in the presence of NaOH gives the lowest glucose concentration 0.45 g/L.

© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/3.0/>).

Peer-review under responsibility of the Organizing Committee of ICCE UNPAR 2013

**Keywords:** empty oil palm fruit bunches; hydrothermal pretreatment; glucose; alkaline; enzymatic hydrolysis.

### 1. Introduction

The production of ethanol has been considered important in solving two modern important issues, i.e. fossil fuel depletion and greenhouse gas problem. Bioethanol produced from biomass comes with extra advantages such as faster octane number, broader flammability limits, higher flame speeds, and higher heats of vaporization. These characteristics allow for a higher compression ratio and shorter burn time, which lead to theoretically efficiency advantages over gasoline in an internal combustion engine [1].

In comparing to the lignocellulosic (LCB) biomass with corn and starch crops, lignocellulosic materials are more preferred to be used as the substrate for the ethanol production. This is due to the expensive price and competition with the human source of foods related factors. LCB material is a plant cell that consists of cellulose, hemicelluloses and lignin. This material is a representative of non-edible biomass: which is renewable, mostly unused and abundantly available source [2].

LCB contains three major components, lignin, cellulose and hemicellulose. Particularly, lignin is constructed by a phenylpropane units linked in a three dimensional structure. Due to this property, lignocellulosic materials are resisting towards cellulosic enzymatic attacks during the fermentation process [2]. Therefore, in order to loosen the tight cross-linked structure and increase the surface area for easier fermentation process a pretreatment of feedstock is required.

The pretreatment of LCB can be categorized into three major groups, physical, chemical and biological pretreatment. Physical pretreatment includes milling, explosion for chemical and physicochemical while fungi used in biological pretreatment [3]. The pretreatment of biomass in ethanol production is important in a way that it enhances the enzyme penetration in enzymatic hydrolysis process. In addition to that, due to the pretreatment process, enzyme is more accessible towards cellulose as the results of decrement in lignin crystallinity and association<sup>4</sup>.

Recently, the usage of water as pretreating agent has shown good results. Basically, biomass is treated with saturated steam pressure (range of pressure) in the presence of water. This treatment has been proven to maximize physical changes and minimize the hydrolysis of cellulose. This has leads to minimal sugar degradation products during pretreatment and thus the pretreated cellulose more reactive for subsequent enzymatic hydrolysis to yield maximum glucose concentration. During the pretreatment, hydronium ions are generated from water and also from acetic acid releases by the hemicellulose. The presence of this hydronium ions is basically autohydrolyzed the linkages of lignocellulosic materials [4].

Unlike to the hydrothermal pretreatment, steam explosion caused not only more hydrolysis of hemicellulose and lignin but also biomass defibration. This happened due to rapid decompression of materials by sudden pressure release during the pretreatment [5].

Hydrothermal pretreatment was known with the added value and of them is the capability of this treatment to use mixed feedstock. Malaysia is among of the country that has lots of natural alkaline material. Therefore in this study, the effect of the alkaline addition on the saccharification efficiencies was studied.

## 2. Materials and methods

### 2.1. Empty fruit bunches

Empty fruit bunches (EFB) were collected from an oil palm mill (FELCRA Nassaruddin Bota) at Perak. The samples were then dried in an electrical oven for 24 h to moisture content below 10% and then grinded and passed through meshed screen of 0.1-0.25 mm size. All samples were kept in an airtight container in order to avoid moisture gain. The samples have been characterized and the basic characteristic of the sample is listed in Table 1.

Table 1: Characteristics of empty fruit bunches.

Property	Value
Moisture dry basis (wt%)	4.14
Hemicellulose (wt%)	24.46
Cellulose (wt%)	49.06
Lignin (wt%)	26.48

### 2.2. Hydrothermal Pretreatment

The hydrothermal pretreatment experiments were carried out in a batch autoclave system (Fig. 1). The sample was heated up in the reactor to a target temperature (Table 2) and then the reactor was cooled to a temperature of 40

°C by using cooling fan. In hydrothermal pretreatment with alkaline addition, 1.2 g each of CaO and NaOH (of R&M Chemicals) was added into the EFB and water mixture. The sample was collected, filtered and stored in a refrigerator before further use.

The product sample was filtered by using vacuum filtration. The solid residue (90% of the recovered solid) was washed twice with 10 mL of distilled water to remove any soluble byproducts that deposited on the EFB surface.

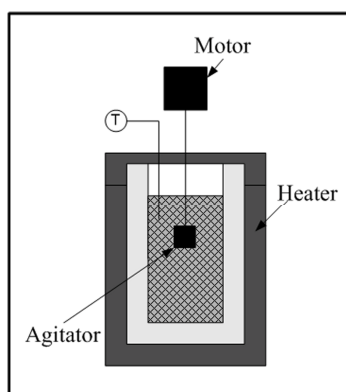


Fig. 1: Autoclave system

Table 2: Hydrothermal pretreatment condition

Run No.	Biomass	Mass of biomass (g)	Volume of water (mL)	Added chemical	Mass of alkaline material (g)	Temperature of treatment (°C)	Reaction time (min)
1.	EFB	30	300	None	0	180	10
2.				CaO	1.2		
3.				NaOH			

### 2.3. Enzymatic hydrolysis

Prior to the filtration process of the hydrothermally pretreated EFB, the solid product was washed twice with distilled water before further used in enzymatic hydrolysis process. The solid residues (2 g of dry basis) of hydrothermally pretreated empty fruit bunches were placed into an Erlenmeyer flask. After that, the enzymes of cellulase from *Aspergillus Niger*, at 40 U/g dry matters (Sigma Aldrich Co.) and 20 U/g dry matter of  $\beta$ -glucosidase extracted from almond (Oriental Yeast Co. Ltd.) were added into the flask. The hydrolysis was conducted in a 50 mM solution citrate buffer of pH 5 giving a dry matter content of 2 wt% of total solution. All of the reaction flasks were put in the shaking incubator (Lab Companion SI-600) and were left for 2 days at 50 °C with a rotating speed of 150 rpm. The samples were collected at every 24h reaction time. The sample was then placed in boiling water to deactivate the enzyme's activity. Finally, the sample was stored in refrigerator before further analyse for glucose content.

### 2.4. Glucose determination

The sample taken was filtered by using micro syringe filters. The glucose produced during hydrolysis was quantified by using High Performance Liquid Chromatograph, HPLC (LC-20, Shimadzu) equipped with an RI detector and amino column (Thermo Scientific) operating at 40 °C with 80% acetonitrile mixture of distilled water as a mobile phase at a flow rate of 1.2 mL/min.

### 3. Results

#### 3.1. Hydrothermal pretreatment

Hydrothermal pretreatment of empty fruit bunches were conducted with and without the addition of alkaline materials, the calcium oxide and sodium hydroxide. Subsequently after the hydrothermal pretreatment process, the products were filtered and stored as wet solid and filtrate. Fig. 2 shows the mass distribution of hydrothermally pretreated EFB. Hydrothermal pretreatment of EFB has lowest portion mass of filtered wet solid (114 g) when compared to the hydrothermal pretreatment with CaO and NaOH addition.

Solid yield of hydrothermally pretreated EFB is shown in Fig. 3. Solid yield ( $Y_s$ ) is the ratio of the mass of the total solid recovered, dry weight basis ( $m_{s,after}$ ) to the mass of the total solid before the reaction ( $m_{s,initial}$ ). Equation 1 shows the definition of this solid yield. The total mass after the reaction was measured once the sample was collected directly after the reactor cooled down to 40 °C. Total mass yield defines the mass recovery of the total reaction. In this figure, the solid yield of the reaction increases significantly for hydrothermal pretreatment with NaOH addition (90.08 %) when compared to the hydrothermal pretreatment of EFB (75.51 %) and with addition of CaO (73.91 %).

$$Y_s (\%) = \frac{m_{s,after}}{m_{s,initial}} \times 100 \quad (1)$$

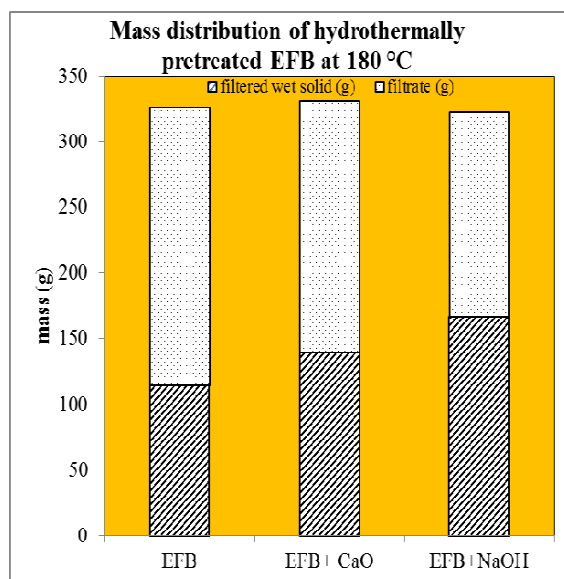


Fig. 2: Mass distribution of hydrothermally pretreated EFB with and without alkaline addition

#### 3.2. Enzymatic hydrolysis

Whatman filter paper No. 1 was used as for reference in the enzymatic hydrolysis process. The hydrolysis of this filter paper produced highest glucose concentration among all substrates (2.0 g/L). In Fig. 4, hydrothermally pretreated EFB without alkaline addition showed a glucose concentration of 0.79 g/L while in the addition of CaO in

hydrothermal pretreatment gave glucose concentration of 0.67 g/L. In contrast, addition of NaOH yielded lower glucose concentration which is 0.45 g/L.

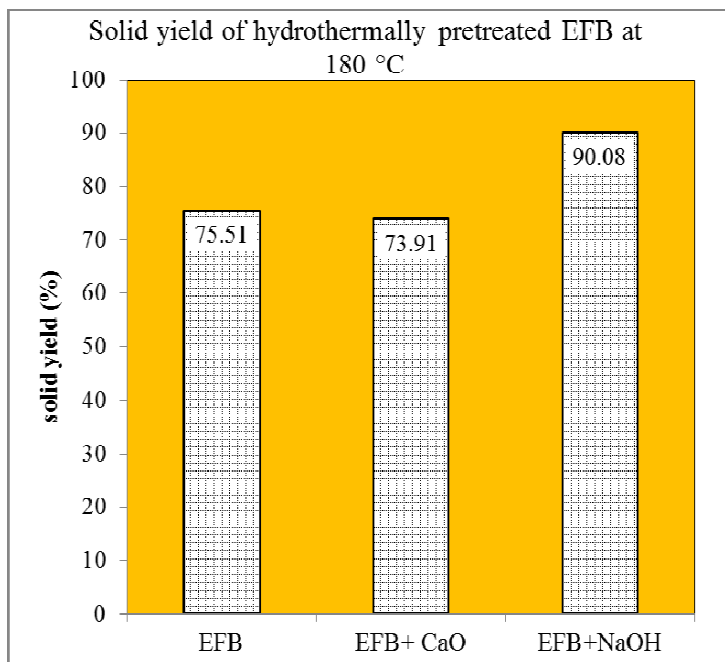


Fig. 3: Solid yield of hydrothermally pretreated EFB with and without alkaline addition

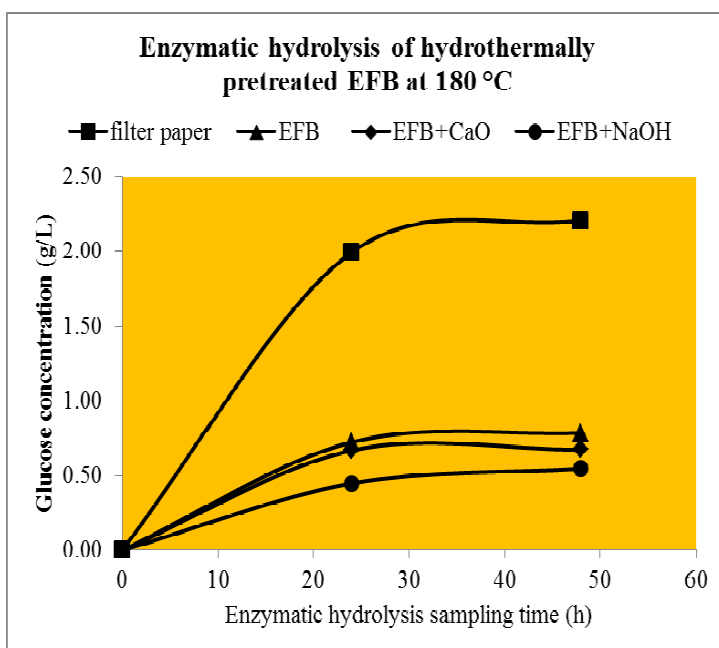


Fig. 4: Glucose concentration (g/L) of enzymatic hydrolysis of hydrothermally pretreated EFB

## 4. Discussion

### 4.1. Hydrothermal Pretreatment

Hydronium ion is a product of water reaction at high temperatures. This ion provides an acidic medium that causes autohydrolysis of EFB linkages in this process. In this work, the pH value recorded for the hydrothermal pretreatment of EFB without alkaline addition was 4.16. In contrast, CaO addition in hydrothermal pretreatment caused the filtrate pH value to increase to 4.64 and becomes less acidic (5.32) in the addition of NaOH.

Besides that, the hydronium ion is also generated at the same time from acetic acid. This acetic acid is actually produced by the thermal splitting of acetyl groups from hemicellulose. The autoionization of this acetic acid due to the high temperature and pressure pushed this chemical to be a catalyst to further promote sugars degradation in the hydrothermal pretreatment process [5].

Basically, addition of alkaline material reduces the acidity of products. In Fig. 2, the mass of the wet solid increases when alkaline material being is into the reaction. This finding is in agreement with Fig. 3. In Fig. 3, when NaOH is being added into the pretreatment process of EFB, the solid yield increases significantly. The increment of this solid suggested that, more linkages are being hydrolyzed by the hydrothermal pretreatment process and led to the increase in production of by-product. This by-product may contain acetyl group of hemicellulose such as acetic acid and phenol groups by the lignin cell based on the black color of the filtrate.

In this hydrothermal pretreatment process, the filtrate from this process is black in color (not shown in this paper). This shows that the hydrothermal pretreatment is capable in altering the linkages, reduces the hemicellulose content and thus increases the accessibility of enzyme penetration in the enzymatic hydrolysis process.

Fig. 2 and Fig. 3 suggest that, more cellulose are accessible for enzyme penetration for the enzymatic hydrolysis process due to increment of the mass of the wet solid and color of the filtrate. This happens due to the solubilization of hemicellulose. Enzymatic saccharification can be improved by the solubilization of hemicellulose [6]. In hydrothermal pretreatment, the hemicellulose solubilization increased as the structure of hemicellulose is more amorphous and less stable compared to cellulose.

### 4.2. Enzymatic hydrolysis

It is expected that, hydrothermal pretreatment with the addition of NaOH yield higher glucose concentration. In contrast, from Fig. 4, hydrothermal pretreatment of the EFB without alkaline addition yields higher glucose concentration. Low glucose concentration of enzymatic hydrolysis from hydrothermally pretreated EFB with alkaline addition may be due to the presence of inhibitory byproducts that contains in the substrate.

These inhibitory byproducts such as acetic acid of acetyl groups from hemicellulose and phenol from furfural group of lignin decrease the enzyme penetration on the surface of the substrate. Therefore, low enzyme penetration yields to low hydrolysis of cellulose into glucose monomers. This finding was highlighted by [7] in which they found that the surface of the pretreated material was covered by phenolic acids which act as a barrier for enzymatic saccharification. Some other studies suggested that enzymatic saccharification could be improved by further increasing accessible area to enhance penetration of more enzymes [4,5,8,9].

## 5. Conclusion

Hydrothermal pretreatment of empty fruit bunches was done with and without alkaline addition at constant temperature, 180 °C and at constant reaction time, 10 min. Following the hydrothermal pretreatment, the solid product recovered from the filtration process was further hydrolyzes enzymatically by using the combination of cellulose and  $\beta$ -glucosidase enzymes. In hydrothermal pretreatment, mass yield of this process in the presence of NaOH gave the lowest value but highest in wet solid mass recovery. It is suggested that, the EFB had more surface area for enzyme penetration but this substrate yielded low glucose concentration in enzymatic hydrolysis process. This might happen due to the presence of the inhibitory byproducts.

## Acknowledgements

This research was made possible through Yayasan Universiti Teknologi PETRONAS (YUTP).

## References

1. Balat M. and Balat H. Recent trends in global production and utilization of bio-ethanol fuel. *Appl Energy*. 2009;**86**(11):2273-82.
2. Taherzadeh M.J. and Karimi K.. Acid-based hydrolysis processes for ethanol from lignocellulosic materials: A review. *BioResources*. 2007; **2**(3):472-99.
3. Gonzalez R., Treasure T., Phillips R., Jameel H. and Saloni D. Economics of cellulosic ethanol production: Green liquor pretreatment for softwood and hardwood, greenfield and repurpose scenarios. *BioResources*. 2011;**6**(3):2551-67.
4. Cybulska I., Lei H. and Julson J. Hydrothermal pretreatment and enzymatic hydrolysis of prairie cord grass. *Energy & Fuels*. 2010: 24:718-27.
5. Kumar S., Kothari U., Kong L., Lee Y.Y. and Gupta R.M. Hydrothermal pretreatment of switchgrass and corn stover for production of ethanol and carbon microspheres. *Biomass Bioenergy*. 2011;**35**(2):956-68.
6. Ruiz H.A., Vicente A.A. and Teixeira J.A. Kinetic modelling of enzymatic saccharification using wheat straw pretreated under autohydrolysis and organosolv process. *Industrial Crops & Products*. 2012;**36**:100-7.
7. Anderson, W.F., Dien B.S., Brandon S.K. and Peterson J.D.. Assessment of Bermuda grass and bunch grasses as feedstock for conversion to ethanol. *Appl. Biochem. Biotechnol*. 2008;**145**:13-21.
8. Liu C. and Wyman C.E. Partial flow of compressed-hot water through corn stover to enhance hemicellulose sugar recovery and enzymatic digestibility of cellulose. *Bioresour. Technol*. 2005;**96**:1978-85.
9. Zhao X., Cheng K. and Liu D. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. *Appl. Biochem. Biotechnol*. 2009;**82**:815-827.